

AD-A065 326

BATTELLE PACIFIC NORTHWEST LABS RICHLAND WASH BIOLOG--ETC F/G 6/18
HEMATOLOGIC EFFECTS IN MICE EXPOSED TO PULSED AND CW MICROWAVES--ETC(U)
FEB 79 H A RAGAN, R D PHILLIPS

N00014-76-C-1154

NL

UNCLASSIFIED

[OF]

AD
A065326



END
DATE
FILMED

4 -79

DDC

AD A0 65326

DDC FILE COPY

Overall title on Contract
Permitted at
code 442
ONR use title on pt

Annual Report

Contract No. N00014-76-C-1154

LEVEL II

EFFECT OF MICROWAVE RADIATION ON PHYSIOLOGICAL AND BEHAVIORAL
FACTORS RELATED TO THE PERFORMANCE CAPABILITY OF LABORATORY ANIMALS

2311103177, Amendment 2

to

Office of Naval Research
Department of the Navy
Arlington, VA 22217



This document has been approved
for public release and sale; its
distribution is unlimited.

Battelle
Pacific Northwest Laboratories
Richland, WA 99352

79 02 12 022

6

HEMATOLOGIC EFFECTS IN MICE EXPOSED TO PULSED AND CW MICROWAVES,

7

Annual rept.,

10

H. A./Ragan
R. D./Phillips

11

February 1979

12 27p.

Work supported by the Office of Naval Research under
Contract No. ~~N00014-76-C-1154~~

15

Biology Department
Pacific Northwest Laboratory
Richland, WA 99352
Operated by
Battelle Memorial Institute

408 910
79 02 12 022 LB

HEMATOLOGIC EFFECTS IN MICE EXPOSED TO PULSED AND CW MICROWAVES

H. A. Ragan
R. D. Phillips

ACCESSION for	
NTIS	White Section <input checked="" type="checkbox"/>
DDC	Buff Section <input type="checkbox"/>
UNANNOUNCED	<i>Per H.A.</i>
JUSTIFICATION	<i>on file</i>
BY	
DISTRIBUTION/AVAILABILITY CODES	
	SPECIAL
<i>A</i>	

Biology Department
Pacific Northwest Laboratory
Richland, WA 99352
Operated by
Battelle Memorial Institute

HEMATOLOGIC EFFECTS IN MICE EXPOSED TO PULSED AND CW MICROWAVES

H. A. Ragan and R. D. Phillips

Experimental Pathology Section and Bioelectromagnetic Program
Biology Department
Battelle
Pacific Northwest Laboratories

ABSTRACT

Mice were exposed in the far field of an anechoic chamber to 2880-MHz pulsed microwaves (2.3-^{microsec} pulses, 100/sec) 3-7.5 hr daily for 60-360 hr. Five studies were performed at average power densities of 5-mW/cm² and four at 10-mW/cm². Mean SARs were 2.25 (5-mW/cm²) and 4.50 (10-mW/cm²) mW/g. An additional group was exposed to 2450-MHz continuous wave (CW) microwaves at a power density of 5-mW/cm². Each group consisted of eight mice, with a concurrently sham-exposed group of eight. In two of five studies with 5-mW/cm² pulsed microwaves there was a significant (~~P < 0.01~~ and ~~< 0.05~~) increase in bone marrow cellularity compared to the sham-exposed groups, but in the other three exposures at 5-mW/cm² no significant effect was observed. Significant differences were occasionally seen in erythrocyte, leukocyte and platelet measurements from microwave-exposed groups, but were not consistently observed. The only effect seen in mice exposed to 5-mW/cm² CW microwaves was a

reduction in reticulocyte concentrations ($P < 0.02$). In one of four exposures at 10-mW/cm^2 pulsed microwaves, mean bone marrow cellularity was reduced ($P < 0.02$) in the microwave-exposed mice, and in another group the concentration of circulating lymphocytes was increased ($P < 0.05$). In only one exposure (10-mW/cm^2 for 360 hr) was any effect noted on serum proteins: a reduction ($P < 0.02$) to 5.1 ± 0.29 g/dl in the exposed vs 5.6 ± 0.36 g/dl in the sham-exposed mice. This was due to a decrease in alpha and beta-globulins, with no effect on albumin or gamma-globulin concentrations. No effect on bone marrow hematopoietic colony-forming units (CFU) was revealed by CFU-agar assay techniques following exposure of mice to 5-mW/cm^2 pulsed microwaves. In 1 of 4 exposures of mice to 10-mW/cm^2 pulsed microwaves there was a significant ($P < 0.05$) increase in CFU-agar colonies. No significant effects of exposure to 10-mW/cm^2 microwaves were observed on assays of in vivo and in vitro cell mediated immune functions. No exposure-related histopathologic lesions were found from examination of several tissues and organs.

INTRODUCTION

The reported effects of microwave exposure on the hematopoietic system are conflicting. Much of this results from the varied animal species and strains used and to the wide diversity of exposure methods. Baranski and Czerski (1) have recently summarized the results of studies involving hematologic effects of microwave exposures of animals.

The present study was undertaken to evaluate the hematologic and immunologic effects of microwave radiation in mice under well-controlled and defined exposure conditions.

MATERIALS AND METHODS

ANIMALS

Female Swiss Webster mice were purchased from a commercial supplier at ~45 days of age and placed in isolation for ~15 days prior to the start of each experiment. For each study sixteen mice were randomly assigned to two groups: eight for microwave exposures and eight for sham exposures. The group assignments were not revealed to the Principal Investigator until after all clinical pathologic parameters had been determined.

EXPOSURE CONDITIONS AND DOSIMETRY

Pulsed microwaves in the 3-GHz (10-cm wavelength) region of the spectrum, within the S-band, were selected for these studies. An APS/20E radar transmitter was provided for use on this project by the U.S. Navy. The unit operates at a frequency of 2.88 GHz with 0.8- or 2.3- μ sec pulse durations at pulse repetition rates of 925 or 308 per second, respectively. By pulsing the transmitter through the external trigger circuit with a time-mark generator (Hewlett-Packard), the 0.8- μ sec duration pulse can be pulsed at 20, 100, 200 or 308 pps. The radar

transmitter was adapted for use in the laboratory to provide for field exposures in an anechoic chamber (2). For this research project animals were exposed to a well-defined plane-wave field in the far field of a transmitter antennae (16.1 dB gain) within an anechoic chamber. The exposure system was calibrated originally using standard antennae range measurements (2), and the accuracy of these determinations were verified by measurements made with an omnidirectional probe and meter (Narda, Model 8305). For CW exposures, an appropriate waveguide was installed to allow powering of the antennae with a continuous wave source. A 2.5-kW microwave source (Varian Model PPS 2.5A) operating at 2450 MHz was available. This provided the means of testing the relative effectiveness of CW exposures at the same average power levels used for pulsed microwaves.

For each study eight mice were individually housed in lucite tubes 10-cm long x 7-cm diameter, with 2-mm-thick walls (Figure 1) and exposed simultaneously to microwave radiation in the far field within the anechoic chamber. The tubes containing the mice were placed on two polyfoam shelves, four mice per shelf (Figure 1), that was located 3.05 meters from the 16.1-dB gain antennae for 5-mW/cm^2 exposures and 2.13 meters for 10-mW/cm^2 exposures. The distance between the tubes was 18 cm. Control mice were handled, housed, and sham-exposed similarly to the exposed mice. In the initial studies they were in a location adjacent to the anechoic chamber that had the same lighting, temperature and relative humidity as the anechoic chamber. For later studies the sham-exposed groups were placed in the anechoic chamber and shielded by

absorbent material during the exposures. Extensive dosimetry in the location of the sham-exposed mice indicated effective shielding from any incident or reflected microwaves.

Power density measurements were made with an omnidirectional probe (Narda, Model 8305) at the eight positions to be occupied by the animals. These measurements were then repeated with all positions occupied with mice in the tubes except at the location where the measurement was being made. To ensure that all animals received the same average dose over the total exposure period, each animal was placed in a new position each day, and thereby rotated through the eight positions over the duration of the exposure period.

Absorbed-dose measurements were made using a twin-well colorimeter as previously described (2). Specific absorption rates (SAR) were calculated from these data for mice exposed parallel to the E, H, and k-vectors. Ambient temperatures and relative humidity were recorded for each dosimetry exposure.

HEMATOLOGY AND SERUM CHEMISTRY

Blood samples for hematologic evaluations were obtained on fasted, lightly ether-anesthetized mice by puncture of the supraorbital plexus. The blood was diluted in isotonic saline and duplicate counts made using a Coulter Model S to provide leukocyte, erythrocyte and hemoglobin concentrations, volume of packed red cells (VPRC), and erythrocyte corpuscular constants (MCV, MCH, MCHC). Blood smears for leukocyte

differential counts were made in duplicate and 100 leukocytes classified from each smear by each of two medical technologists. Reticulocytes were stained using new methylene blue and enumerated by determining the number of reticulocytes in a minimum of 1000 erythrocytes. Platelets were counted using standard methods under phase microscopy. Red cell size distribution (volume histogram) was determined using a Coulter Model ZH coupled to a multichannel analyzer and X-Y plotter.

To determine bone marrow cellularity a femur was debrided and flushed repeatedly with 1.0 ml of tissue culture media. Dilutions were made, lysed to remove intact erythrocytes, and the number of nucleated bone marrow cells enumerated using a Coulter Model ZH. The contralateral femur was removed to obtain bone marrow impression smears for cytologic evaluation. In addition, bone marrow and spleen sections were stained using Prussian blue for subsequent evaluation of tissue iron stores.

Bone marrow myelocytic stem cells were quantitated in vitro using a modification of the soft-agar technique (4). Mouse postendotoxin-serum or an extract of pregnant mouse uteri was used as the colony-stimulating factor(s). For these studies 50×10^3 nucleated bone marrow cells were cultured and the colonies counted after 7 days in culture. Bone marrow stem cells were quantitated in vivo by the method of Till and McCulloch (5). Briefly, 50×10^3 nucleated bone marrow cells from each microwave- or sham-exposed mouse were injected into mice lethally irradiated using a cobalt-60 source and, 8 days later the spleens were removed, weighed, and placed in Bouin's fixative prior to enumeration of the spleen colonies.

Serum for clinical chemistry parameters was obtained via heart puncture from anesthetized mice. Serum protein concentrations were determined using an automated biuret method.^(a) Serum proteins were quantitated after fractionation by electrophoresis on cellulose acetate strips and densitometric quantitation. Serum triglyceride concentrations were evaluated by the enzymatic method of Hycel.^(b)

IMMUNOLOGY

The cell-mediated arm of the immune system was evaluated by determining the response to keyhole limpet hemocyanin (KLH), and contact sensitivity to dinitrofluorobenzene (DNFB). Mice were immunized by subcutaneous injection of 1 mg KLH in Freund's complete adjuvant 13 days prior to the termination of exposures. Following the last exposure they were challenged by injection of 20 μ l KLH in the shaved flank. The measure of response was the increase in skin thickness over the pre-injection value (6).

For DNFB sensitization, 0.5% DNFB in olive oil/acetone was painted on the shaved abdomen on 2 successive days. Four days later the ears were measured with a micrometer and a challenge dose of DNFB was painted on the dorsal side of the ear. The response was assayed 24 hr later as the increase in ear thickness compared with that prior to the challenge dose (7).

In vitro cell-mediated immunity studies were conducted using mitogen stimulated incorporation of 125 I-Iododeoxyuridine (IUDR) into spleen cell

^a AutoAnalyzer II, Technicon Instruments, Tarrytown, NY
^b Hycel Inc., Houston, TX

cultures as an indicator of lymphocyte responsiveness. The cells were cultured in the presence of the mitogen for 2 days and pulsed for the last 20 hr with 2.0 μCi of IUDR, lysed and washed with water and acetic acid. Coupled with this study was in vitro KLH stimulation of the spleen cell preparations from mice immunized previously with KLH (8,9).

STATISTICS

Mean values and standard deviations were calculated by standard methods, and group means compared using Student's two-tailed t test.

RESULTS

DOSIMETRY

Power density measurements for the nominal 10-mW/cm² exposures made at the eight positions when empty had a mean value of 9.9-mW/cm², and ranged from 7.8-11.6-mW/cm² over the eight positions. When these measurements were repeated with mice in all positions except that position being measured, the average power density then was 10.2-mW/cm², and ranged from 6.7-13.6-mW/cm² over the eight positions. Similar measurements were made for the nominal 5-mW/cm² exposures, and with mice in the field the average power density for the eight positions was 5.0-mW/cm², and ranged from 3.2-6.5-mW/cm² over the eight positions.

Specific absorption rates (SAR) were calculated from data using the twin-well calorimeter following exposures. Seven separate exposures

were made on different days for dosimetry at each vector orientation. Mean ambient temperature for these exposures was 21.7 ± 0.4 C, and relative humidity $42 \pm 2\%$. Mean SAR values for each orientation were: E-vector 0.63 ± 0.04 , H-vector 0.34 ± 0.02 , and k-vector 0.37 ± 0.02 mW/g/unit power density (\pm SEM), or a mean SAR considering all vectors of 0.45 ± 0.03 mW/gm/unit power density.

Air flow in the 58,200 liter anechoic chamber was 12,500-14,200 l/m, resulting in about ~ 15 air changes hourly. Ambient temperature during a seven hour exposure at 10-mW/cm^2 increased ~ 1.8 C, e.g., during a 51 day exposure, seven hours daily, initial temperatures were 22.05 ± 1.02 C and at the end of the day were 23.85 ± 0.83 C (mean ± 1 standard deviation).

HEMATOLOGY AND SERUM CHEMISTRY

Hematologic and serum chemistry evaluations of mice exposed to 5-mW/cm^2 for our experiments are shown in Table 1. Experiment #7 was primarily for serum chemistry studies, but femoral marrow cellularity was also determined. Parameters for which statistically significant differences were noted between the exposed and sham-exposed mice are shown in Table 2. The total exposure at 5-mW/cm^2 was 75-80 hr (7-8 hr/day for ~ 10 days). In two of the four experiments femoral marrow cellularity was significantly increased in the microwave exposed mice as compared to their sham-exposed controls. No other consistent effects on hematologic or serum chemistry parameters were observed. One exposure (Experiment #4) was to CW microwaves for 75 hr total exposure (7.5 hr/day for 10 days). The only effect noted was a reduced reticulocyte concentration in the microwave exposed group.

Data for six experiments at 10-mW/cm^2 power density are shown in Table 3. Total exposures varied from 60-360 hr (7-8 hr/day). Parameters with significant differences between the exposed and sham-exposed mice are shown in Table 4. There were no reproducible differences observed between the microwave and sham-exposed mice.

HEMATOPOIETIC STEM CELL ASSAYS

Quantitations of the bone marrow myeloid stem-cell compartment as assayed using the in vitro methylcellulose culture method are shown in Table 5. The results shown for each experiment are mean values of colonies greater than 50 cells obtained from 5-8 mice per group with four culture plates made from the marrow of each mouse. There is a large ($\sim 40\%$) coefficient of variation which is more a reflection of variability among animals than between plates for individual mice. However, with the exception of Experiment #13 no significant differences were observed between the microwave-exposed and sham-exposed mice.

Quantitation of the pluripotential hematopoietic stem cell was attempted using the in vivo colony-forming-unit spleen assay in Experiments #12 and #13. For each study, marrow from each of five microwave-exposed or sham-exposed mice was injected into five recipient mice that were killed either 10 (Experiment #12) or 7 (Experiment #13) days later. In Experiment #12 hematopoietic cell colonies observed on the surface of spleens from the recipient mice were 6.0 ± 3.3 and 8.0 ± 5.3 from the exposed and sham groups, respectively. In Experiment #13 the recipient

mice were killed only 7 days after marrow injection in an attempt to more accurately quantitate total splenic colonies microscopically (rather than only those visible on the surface, as done in Experiment #12). This, however, was unsuccessful since the spleen colonies were confluent and could not be counted accurately.

IMMUNE ASSAYS

The in vivo cell-mediated immune response was evaluated in eight mice per group in two experiments (#10-A and #14) following pulsed microwave exposures to 2880 MHz 3 hr daily for 20 days at a power density of 10-mW/cm². The results are shown in Table 6. There were no significant differences between treatment groups.

The delayed hypersensitivity response is intimately dependent on the interaction of leukocytes. Therefore, in Experiment #14 blood samples were obtained to examine the leukocyte and serum protein responses at the time the cell-mediated immune response was evaluated. The results are shown in Table 7. No differences were observed in the cellular responses or serum protein fractions between the exposed and sham-exposed mice.

The cell-mediated immune response was also evaluated in vitro as the ability of spleen cells to enter DNA synthesis after exposure to different T and B lymphocyte mitogens. The results are shown in Table 8. There was considerable variability between mice from both groups in the response to the various mitogens. There were, however, no significant

differences noted between the exposed and sham-exposed mice, and the coefficients of variation of mitogenic responses were similar for each group. (These mitogen studies were performed by Dr. J. E. Morris).

PATHOLOGY

Extensive tissues were taken from mice exposed to 10-mW/cm^2 pulsed microwaves in two studies. Exposures were for 200 and 360 hr. Tissues examined by light microscopy included liver, spleen, kidneys, adrenals, postcervical lymph node, thymus, heart, lung, brain, eyes, femoral bone and marrow. There were no histologic changes observed in the exposed mice that could be related to microwave exposure when compared with tissues from the sham-exposed groups. (Histopathologic evaluations were made by Dr. R. H. Busch).

CONCLUSIONS

From the results of these studies following numerous exposures of mice to well-defined pulsed microwave fields with defined dosimetry there were no consistent effects observed at 5- or 10-mW/cm^2 exposures 3-7 hr daily for several weeks. The only exception was an increased bone marrow cellularity at 5-mW/cm^2 which was significantly greater in the exposed mice of two studies and greater, although not significantly so, in a third study.

The failure to detect any consistent hematologic or immunologic effects of microwave exposure are contrary to those reported by Eastern

European investigators using power densities at least 10-fold lower than employed in these studies (1). An explanation for these differences in effects is not readily apparent. We used a horizontal field, whereas some other studies were in a vertical field array. Our animals were housed individually during exposure with spacing to minimize field enhancement or attenuation, whereas some of the earlier studies of the European scientists used animals housed multiply; and many of the previous studies were prior to the development of precise absorbed-dose measurements. Finally, one must consider a "power-window" effect in which case one might not achieve a dose-response curve.

REFERENCES

1. Baranski, S. and P. Czerski. Biological Effects of Microwaves. Danden, Hutchinson and Ross, Inc. Stroudsburg, PA, pp. 137-146, 1976.
2. Phillips, R. D., E. L. Hunt and N. W. King. Field measurements, absorbed dose, and biological dosimetry of microwaves. Ann. N.Y. Acad. Sci. 247: 499-511, 1975.
3. Gandhi, O. P. Conditions of strongest electromagnetic power deposition in man and animals. IEEE Trans. on Microwave Theory and Techniques MTT-23: 1021-1029, 1975.
4. Bradley, T. R. and D. Metcalf. The growth of mouse bone marrow cells in vitro. Austr. J. Exp. Biol. Med. 44: 287-300, 1966.
5. Till, J. E. and E. A. McCulloch. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Rad. Res. 14: 213-222, 1961.
6. Temple, A. and G. Loewi. Transfer of delayed hypersensitivity in rats. Immunology 20: 799-802, 1971.
7. Phanuphak, P., J. W. Moorhead and H. N. Claman. Tolerance and contact sensitivity to DNFB in mice. J. Immunol. 112: 849-851, 1974.
8. Cohen, A. M., J. F. Burdick and A. S. Ketcham. Cell-mediated cytotoxicity: An assay using ^{125}I -iododeoxyuridine-labeled target cells. J. Immunol. 107: 895-898, 1971.

9. Oppenheim, J. J. and D. L. Rosenstreich. Lymphocyte transformation: Utilization of automatic harvesters, Chapter 54. In: In Vitro Methods in Cell-Mediated and Tumor Immunity, B. R. Bloom and J. R. David (Eds.). Academic Press, New York, NY, 1976.

TABLE 1. Hematologic Evaluations in Mice Exposed to 2880-MHz Pulsed Microwaves at a Power Density of 5-mW/cm² (SAR = 2.25 mW/g) [Mean Values \pm 1 SD]

	Experiment #1		Experiment #5		Experiment #6		Experiment #7	
	Exposed	Sham	Exposed	Sham	Exposed	Sham	Exposed	Sham
Hours/Day	7.0	0	8.0	0	7.5	0	7.5	0
Total Hours	76	0	80	0	75	0	75	0
Killed After Exposure (hr)	90	90	90	90	16	16	16	16
WPC ml/dl	43.5 \pm 1.2	43.5 \pm 1.5	38.0 \pm 1.7	38.8 \pm 1.2	37.6 \pm 1.6	37.8 \pm 1.0	---	---
RBC $\times 10^6/\mu\text{l}$	9.93 \pm 0.24 ^a	9.39 \pm 0.50	8.05 \pm 0.49	8.15 \pm 0.35	7.94 \pm 0.48	7.91 \pm 0.21	---	---
Hbg g/dl	15.2 \pm 0.5	15.1 \pm 0.6	15.9 \pm 0.6	16.2 \pm 0.6	14.2 \pm 0.8	14.1 \pm 0.4	---	---
MCV μl	42.0 \pm 1.0 ^a	45.0 \pm 3.0	47.0 \pm 0.2	48.0 \pm 2.0	48.0 \pm 1.0	48.0 \pm 2.0	---	---
MCH μg	15.7 \pm 0.3	16.4 \pm 0.6	19.3 \pm 0.7	19.4 \pm 0.6	17.9 \pm 0.4	17.8 \pm 0.5	---	---
MCHC %	36.0 \pm 1.0	36.0 \pm 1.0	41.0 \pm 1.0	41.0 \pm 1.0	38.0 \pm 1.0	38.0 \pm 1.0	---	---
Reticulocytes $\times 10^3/\mu\text{l}$	210 \pm 74	335 \pm 162	76 \pm 21 ^c	109 \pm 35	100 \pm 37	92 \pm 50	---	---
WBC $\times 10^3/\mu\text{l}$	3.0 \pm 1.2	3.2 \pm 1.1	10.4 \pm 3.2	11.7 \pm 2.3	5.8 \pm 1.7	5.5 \pm 1.8	---	---
Neutrophils $\times 10^3/\mu\text{l}$	0.22 \pm 0.10	0.29 \pm 0.25	1.32 \pm 0.90	1.69 \pm 0.45	0.43 \pm 0.18	0.44 \pm 0.18	---	---
Lymphocytes $\times 10^3/\mu\text{l}$	2.77 \pm 1.17	2.85 \pm 0.97	8.65 \pm 2.76	9.51 \pm 1.79	5.18 \pm 1.57	4.96 \pm 1.73	---	---
Monocytes $\times 10^3/\mu\text{l}$	0.02 \pm 0.02	0.01 \pm 0.02	0.19 \pm 0.08	0.26 \pm 0.12	0.04 \pm 0.05	0.06 \pm 0.04	---	---
Eosinophils $\times 10^3/\mu\text{l}$	0.03 \pm 0.02	0.04 \pm 0.04	0.25 \pm 0.19	0.21 \pm 0.18	0.11 \pm 0.07	0.08 \pm 0.04	---	---
Platelets $\times 10^3/\mu\text{l}$	687 \pm 212	976 \pm 247	849 \pm 301	913 \pm 162	885 \pm 108	845 \pm 142	---	---
Femoral Marrow $\times 10^6$	15.4 \pm 1.5 ^c	11.9 \pm 1.4	19.2 \pm 4.5	16.5 \pm 3.5	16.8 \pm 3.6 ^a	13.2 \pm 2.4	17.2 \pm 2.5	18.8 \pm 4.2
Total Protein g/dl	5.8 \pm 0.3	5.6 \pm 0.4	---	---	---	---	6.7 \pm 0.6	6.3 \pm 0.3
Albumin %	63.0 \pm 2.0	62.0 \pm 5.0	50.0 \pm 3.0	53.0 \pm 3.0	54.0 \pm 4.0	51.0 \pm 3.0	57.0 \pm 3.0	57.0 \pm 2.0
α Globulins %	14.0 \pm 1.0	14.0 \pm 2.0	19.0 \pm 2.0	18.0 \pm 2.0	16.0 \pm 1.0	17.0 \pm 1.0	17.0 \pm 1.0	18.0 \pm 1.0
β Globulins %	16.0 \pm 2.0	16.0 \pm 3.0	21.0 \pm 1.0	20.0 \pm 4.0	22.0 \pm 4.0	23.0 \pm 2.0	17.0 \pm 2.0	17.0 \pm 2.0
γ Globulins %	7.0 \pm 1.0	8.0 \pm 2.0	10.0 \pm 2.0	9.0 \pm 2.0	7.0 \pm 1.0	8.0 \pm 2.0	10.0 \pm 2.0	9.0 \pm 2.0
Triglycerides mg/dl	141 \pm 15 ^a	114 \pm 9	148 \pm 27	148 \pm 24	190 \pm 38	160 \pm 20	157 \pm 39	162 \pm 18
Body Weight g	---	---	---	---	---	---	---	---
Spleen Weight mg	---	---	137 \pm 22	142 \pm 19	124 \pm 20 ^a	100 \pm 19	---	---

^a $p < 0.05$

^b $p < 0.01$

^c $p < 0.001$

TABLE 2. Statistically Significant Differences Between Mice Exposed to 5-mW/cm² Microwaves and Sham-Exposed Mice

Experiment Number	Hours	Parameter	Exposed	Sham-Exposed	P<
1-PW	75	Erythrocytes x 10 ⁶ /μl	9.93	9.39	0.02
		Platelets x 10 ³ /μl	687	976	0.05
		Femoral Marrow x 10 ⁶	15.4	11.9	0.001
		Triglycerides mg/dl	141	114	0.05
5-PW	80	Reticulocytes x 10 ³ /μl	76	109	0.001
6-PW	75	Femoral Marrow x 10 ⁶	16.8	13.2	0.05
		Spleen mg	124	100	0.05
4-CW	75	Reticulocytes x 10 ³ /μl	104	154	0.02

TABLE 3. Hematologic Evaluations in Mice Exposed to 2000 MHz Pulsed Microwaves at a Power Density of 10-mW/cm² (SAR = 4.50 mW/g) [Mean Values ± 1 SD]

	Experiment #8		Experiment #9		Experiment #10		Experiment #11		Experiment #12		Experiment #13	
	Exposed	Sham	Exposed	Sham	Exposed	Sham	Exposed	Sham	Exposed	Sham	Exposed	Sham
Hours/Day	7.5	0	3	0	0	0	7	0	7	0	7	0
Total hours	75	0	60	0	0	0	180	0	360	0	200	0
Killed After Exposure (hr)	90	90	16	16	16	16	16	16	16	16	16	16
APC m/dl	37.2 ± 1.9	39.2 ± 1.2	34.1 ± 3.8	34.2 ± 2.5	37.8 ± 1.5	39.0 ± 1.7	42.2 ± 1.9	41.7 ± 1.8	39.9 ± 4.4	42.4 ± 1.0	38.3 ± 1.7 ^a	36.5 ± 1.7
RBC x 10 ⁶ /μl	8.04 ± 0.51	8.47 ± 0.37	7.23 ± 0.67	7.28 ± 0.56	8.18 ± 0.52	8.27 ± 0.32	9.05 ± 0.43	8.93 ± 0.41	8.27 ± 0.96	8.79 ± 0.43	8.08 ± 0.33 ^a	7.72 ± 0.34
Hgb g/dl	14.0 ± 0.9	15.1 ± 0.5	13.2 ± 1.4	13.4 ± 1.0	14.3 ± 0.7	14.5 ± 0.7	15.2 ± 0.7	15.1 ± 0.5	14.5 ± 1.7	15.4 ± 0.5	14.5 ± 0.5	14.0 ± 0.6
MCV μ ³	48.0 ± 1.0	48.0 ± 1.0	47.0 ± 2.0	47.0 ± 1.0	47.0 ± 2.0	48.0 ± 2.0	47.0 ± 1.0	48.0 ± 1.0	49.0 ± 1.0	49.0 ± 2.0	48.0 ± 1.0	47.0 ± 1.0
MCH μg	17.6 ± 0.5	17.9 ± 0.3	18.5 ± 0.5	18.5 ± 0.5	17.7 ± 0.4	17.7 ± 0.7	17.1 ± 0.8	17.3 ± 0.4	17.5 ± 0.4	17.5 ± 0.8	18.0 ± 0.4	18.2 ± 0.3
MCHC %	38.0 ± 1.0	39.0 ± 1.0	40.0 ± 1.0	40.0 ± 1.0	38.3 ± 0.6	37.7 ± 0.7	36.0 ± 1.0	36.0 ± 1.0	37.1 ± 0.5	37.2 ± 0.7	38.1 ± 0.4	38.6 ± 0.4
Reticulocytes x 10 ³ /μl	175 ± 108	191 ± 62	159 ± 90	99 ± 76	193 ± 71	198 ± 56	---	---	167 ± 45	152 ± 64	207 ± 72	144 ± 53
WBC x 10 ³ /μl	4.2 ± 0.8	4.2 ± 0.9	5.5 ± 0.9	4.9 ± 1.0	4.8 ± 1.4	4.4 ± 1.6	3.9 ± 1.0	4.6 ± 1.2	5.1 ± 0.7	5.7 ± 0.8	6.7 ± 1.1	7.0 ± 1.6
Neutrophils x 10 ³ /μl	0.62 ± 0.29	0.48 ± 0.21	0.46 ± 0.27	0.55 ± 0.17	0.63 ± 0.29	0.62 ± 0.28	0.46 ± 0.20	0.63 ± 0.27	0.68 ± 0.38	0.93 ± 0.41	0.93 ± 0.23	0.80 ± 0.18
Lymphocytes x 10 ³ /μl	3.54 ± 0.73	3.67 ± 0.79	5.03 ± 0.89 ^a	4.34 ± 0.89	4.04 ± 1.4	3.76 ± 1.4	3.31 ± 0.80	3.93 ± 1.18	4.34 ± 0.69	4.70 ± 0.78	5.56 ± 1.08	5.96 ± 1.59
Monocytes x 10 ³ /μl	0.03 ± 0.03	0.03 ± 0.02	0.01 ± 0.03	0.01 ± 0.01	0	0	0.06 ± 0.07	0.04 ± 0.04	0.02 ± 0.04	0.02 ± 0.02	0.04 ± 0.04	0.01 ± 0.01
Eosinophils x 10 ³ /μl	0.04 ± 0.05	0.04 ± 0.03	0.01 ± 0.02	0.05 ± 0.04	0.04 ± 0.05	0.03 ± 0.05	0.02 ± 0.02	0.02 ± 0.02	0.06 ± 0.03	0.04 ± 0.04	0.16 ± 0.12	0.20 ± 0.06
Platelets x 10 ³ /μl	1172 ± 259	1000 ± 268	788 ± 255	882 ± 192	1019 ± 237	948 ± 136	---	---	1038 ± 160	1038 ± 205	905 ± 289	908 ± 189
Platelets x 10 ⁶ /mm ³	14.8 ± 1.0 ^b	20.4 ± 5.3	18.6 ± 2.5	19.8 ± 3.0	24.7 ± 5.5	26.3 ± 1.7	19.6 ± 5.0	20.6 ± 6.7	22.2 ± 6.6	23.7 ± 3.2	13.4 ± 1.4	14.6 ± 2.1
Total Protein g/dl	---	---	4.8 ± 0.34	4.8 ± 0.35	5.0 ± 0.30	4.9 ± 0.27	---	---	5.1 ± 0.29 ^b	5.6 ± 0.36	5.6 ± 0.57	5.8 ± 0.39
Albumin %	50.0 ± 4.0	51.0 ± 3.0	52.0 ± 2.0	54.0 ± 4.0	50.0 ± 5.0	49.0 ± 4.0	---	---	62.0 ± 5.0	58.0 ± 3.0	56.0 ± 3.0	58.0 ± 2.0
α Globulin %	26.0 ± 2.0	26.0 ± 3.0	16.0 ± 1.0	16.0 ± 2.0	20.0 ± 3.0	21.0 ± 2.0	---	---	14.0 ± 3.0 ^b	16.0 ± 2.0	18.0 ± 2.0	16.0 ± 1.0
β Globulin %	17.0 ± 1.0	16.0 ± 1.0	21.0 ± 2.0	20.0 ± 2.0	18.0 ± 1.0	17.0 ± 1.0	---	---	16.0 ± 2.0 ^c	17.0 ± 1.0	16.0 ± 2.0	17.0 ± 1.0
γ Globulin %	7.0 ± 2.0	7.0 ± 1.0	10.0 ± 2.0	10.0 ± 1.0	12.0 ± 2.0	12.0 ± 2.0	---	---	8.0 ± 2.0	8.0 ± 2.0	10.0 ± 2.0	9.0 ± 2.0
Triglycerides %	122 ± 33	103 ± 14	129 ± 61	122 ± 25	111 ± 13	98 ± 19	---	---	124 ± 26	106 ± 18	73 ± 38 ^a	39 ± 19
Body Weight g	---	---	29.0 ± 1.0	29.0 ± 1.0	26.0 ± 1.0	25.0 ± 2.0	25.4 ± 1.8	24.7 ± 1.5	26.0 ± 1.0	25.0 ± 2.0	29.0 ± 2.0	29.0 ± 2.0
Spleen Weight mg	128 ± 16 ^c	103 ± 10	135 ± 19	137 ± 20	101 ± 16	107 ± 20	85 ± 18	90 ± 18	103 ± 15	92 ± 20	132 ± 23	127 ± 18

^a P < 0.05
^b P < 0.02
^c P < 0.001

TABLE 4. Statistically Significant Differences
Between Mice Exposed to 10-mW/cm²
Pulsed Microwaves and Sham-Exposed
Mice

Experiment Number	Hours	Parameter	Exposed	Sham-Exposed	P<
8-PW	75	Femoral Marrow x 10 ⁶	14.8	20.4	0.02
		Spleen mg	128	103	0.01
9-PW	60	Lymphocytes x 10 ³ /μl	5.03	4.34	0.05
12-PW	360	Protein g/dl	5.12	5.62	0.02
		α Globulins g/dl	0.73	0.92	0.02
		β Globulins g/dl	0.79	0.96	0.01
		Thymus mg	58	44	0.01
13-PW	200	VPRC ml/dl	38.3	36.5	0.05
		Erythrocytes x 10 ⁶ /μl	8.08	7.72	0.05
		CFUc Colonies	37	28	0.05
		Triglycerides mg/dl	73	39	0.05

TABLE 5. Bone Marrow Colony-Forming-Units
In Vitro from Microwave Exposed
and Sham-Exposed Mice (Mean \pm 1 SD)

<u>Experiment No.</u>	<u>Microwave</u>	<u>Power Density</u>	<u>Exposed</u>	<u>Sham</u>	<u>P <</u>
4	CW	5	38.4 \pm 12.6	36.2 \pm 13.9	NS
6	PW	5	27.8 \pm 10.4	31.4 \pm 10.3	NS
7	PW	5	23.9 \pm 18.1	22.8 \pm 8.9	NS
10	PW	10	20.4 \pm 10.1	17.7 \pm 7.7	NS
11	PW	10	26.1 \pm 9.9	22.3 \pm 5.7	NS
12	PW	10	12.9 \pm 3.70	17.0 \pm 19.2	NS
13	PW	10	37.0 \pm 15.0	27.7 \pm 6.1	0.05

TABLE 6. Cell-Mediated Immune Response of Mice Exposed to 10-mW/cm²
Pulsed Microwaves 3 Hr Daily for 20 Days (Mean \pm 1 SD,
n=8/group)

Experiment Number	Group	KLH Response Δ Increase in Skin Thickness (μ m)	DNFB Response Δ Increase in Ear Thickness (μ m)
10-A	Exposed	53 \pm 109	256 \pm 53
	Sham-Exposed	148 \pm 76	196 \pm 38
14	Exposed	152 \pm 81	109 \pm 66
	Sham-Exposed	127 \pm 53	119 \pm 66
	Unsensitized Controls	60 \pm 58	10 \pm 18

TABLE 7. Leukocyte and Serum Protein Values from Mice Exposed to 10-mW/cm² Pulsed Microwaves 3 Hr Daily for 20 Days (Mean \pm 1 SD, n=8/group)

Group	WBC $\times 10^3/\mu\text{l}$	Neutrophils $\times 10^3/\mu\text{l}$	Lymphocytes $\times 10^3/\mu\text{l}$	Monocytes $\times 10^3/\mu\text{l}$	Eosinophils $\times 10^3/\mu\text{l}$	Serum Protein g/dl	Albumin g/dl	Alpha Globulins g/dl	Beta Globulins g/dl	Gamma Globulins g/dl
Exposed	8.9 \pm 1.4	2.3 \pm 1.1	6.3 \pm 1.0	0.18 \pm 0.08	0.18 \pm 0.10	4.9 \pm 0.4	2.45 \pm 0.22	0.87 \pm 0.13	1.06 \pm 0.13	0.48 \pm 0.07
Sham-Exposed	9.5 \pm 3.1	3.1 \pm 1.2	6.0 \pm 2.1	0.26 \pm 0.17	0.21 \pm 0.13	5.1 \pm 0.6	2.52 \pm 0.29	0.98 \pm 0.11	1.10 \pm 0.17	0.50 \pm 0.09
Unsensitized Controls	9.1 \pm 2.4	1.2 \pm 0.5	7.5 \pm 1.9	0.06 \pm 0.06	0.30 \pm 0.20	5.2 \pm 0.6	2.78 \pm 0.52	0.91 \pm 0.07	0.94 \pm 0.12	0.59 \pm 0.08

TABLE 8. Stimulation Index¹ of Spleen Cells to Various Mitogens
(Mean \pm 1 SD, n=6/group). Mice exposed to 10-mW/cm²
3 hr daily for 20 days.

<u>Mitogen</u>	<u>Exposed Mice</u>	<u>Sham-Exposed Mice</u>
Concanavalin A	23.3 \pm 14.0	22.4 \pm 7.9
Lipopolysaccharide	6.3 \pm 5.2	5.5 \pm 3.5
Poke Weed Mitogen	3.7 \pm 2.0	4.8 \pm 2.3
Phytohemagglutinin	24.5 \pm 33.9	22.8 \pm 29.8
Keyhole Limpet Hemocyanin	10.2 \pm 9.2	10.9 \pm 10.5

$$^1 \text{ Stimulation index} = \frac{\text{CPM after mitogen exposure}}{\text{CPM of unstimulated sample}}$$

